Corn Cell Wall Peroxidases: Potential Role in the Lignification Process

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Introduction

In general, plants produce several isozymes of peroxidase. Corn cell walls contain at least 12 to 15 different isozymes with peroxidase activity. The role of these isozymes in plant development is not clear. We have investigated three major groups of peroxidases isolated and partially purified from the walls of corn suspension culture cells. Previous work suggested that all peroxidases were equally adept at forming dehydrodimers of ferulate model compounds and that there was no difference in the types of dimers formed. We have now extended this work to investigate the potential role in the formation of lignin polymers.

Materials and Methods

Peroxidases were isolated from the walls of corn suspension cultures and partially purified using DEAE anion exchange and chromatofocusing chromatography. Three major groups, differing in their isoelectric points, were used in these studies (group I pI 9-10, group II pI 7-8, and group III pI 3-5). For comparison, peroxidases were also extracted from the apoplastic space of corn stem segments using gentle centrifugation (700 Xg) and a 20 mM acetate buffer (pH 5.0 200 mM $CaCl_2$). After removing apoplastic materials, stem segments were homogenized in cold (4 °C) 50 mM acetate buffers and the walls collected after washing with cold buffer, cold acetone (-20 °C), and again with cold buffer. Walls were suspended in 200 mM $CaCl_2$ overnight to extract wall bound peroxidases. Peroxidase activity was evaluated using ethyl ferulate (Et-FA), methyl *p*-coumarate (Me-*p*CA), coniferyl alcohol (CA), and sinapyl alcohol (SA). All activity reactions (total volume 1 mL) were run in acetate or MES buffers (pH 5.0) containing the appropriate substrate (0.14 to 0.16 µmoles) with hydrogen peroxide (10 µL of 7.5 mM solution) added to initiate the reaction. Rates of reactions were determined by continuously monitoring (total of 5 min) the change in absorbance at the appropriate wavelength for each substrate (ethyl ferualte, 320 nm; methyl *p*-coumarate, 308 nm; coniferyl alcohol, 261 nm; and sinapyl alcohol, 270 nm).

Results and Discussion

Peroxidase (POD) activity extracted from corn suspension cells varied with the type of substrate and the pI group (Table 1). Generally for all POD groups, the order of substrate reactivities was Et-FA > Me-*p*CA $\geq$ CA >> SA. Only Group I POD had a reasonable activity against sinapyl alcohol; all others were 20 to 40 times less active.

A comparison of POD activity extracted from corn stem walls was different from the suspension cultures in substrate preference such that Et-FA > CA > Me-*p*CA >> SA (Table 2). We evaluated different regions (upper and lower) within stem internodes and progressively more mature internodes (Int 2 = second fully expanded internode above the soil line, Int 5 = fifth internode, Int 7 = seventh internode, least mature). For grasses, like corn, the upper portion of the internode is the most mature part and the lower less mature. Substrates Et-FA and CA showed clear trends of increased activity with maturity while Me-*p*CA and SA did not reveal consistent trends with maturity. The $CaCl_2$ extracts of stem walls after homogenization showed the same activity trends as the centrifugation method (data not shown).

We had thought that perhaps the more mature portions of the stem might produce POD enzymes having higher specificity for sinapyl alcohol since, as the stem matures, there is an increase in the sinapyl monomers incorporated into lignin. This was clearly not the case (Table 2). We tested a hypothesis proposed by Takahama et al. (1996) that hydroxycinnamic acids may aid in the incorporation of sinapyl alcohol monomers into lignin. The addition of as

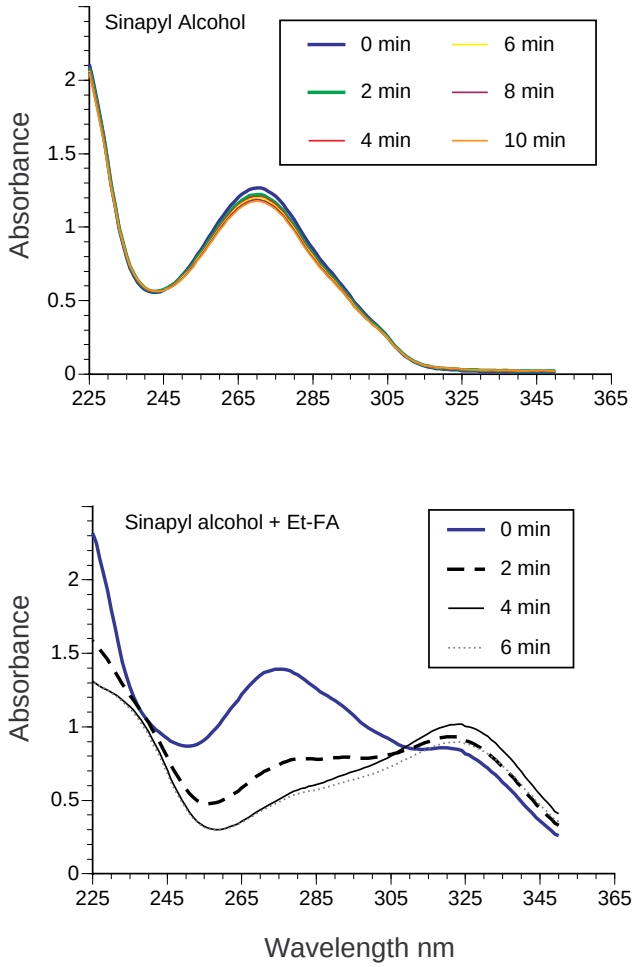

Figure 1. Spectral changes of sinapyl alcohol during coupling reactions mediated by cell wall peroxidases. A. Sinapyl alcohol with POD Group I and H_2O_2 B. Same as A except 0.045 µmoles of ethyl ferulate was included in the reaction mixture.

Table 1. Comparison of POD isozymes isolated from corn suspension culture walls. Specific acitivity towards different substrates (ethyl ferulate, Et-FA; methyl *p*-coumarate, Me-*p*CA; coniferyl alcohol, CA; and sinapyl alcohol, SA).

	Specific activity (µmoles/mg/sec)			
Isozyme Group	Et-FA	Me-*p*CA	CA	SA
Group I	12.85	8.33	5.12	1.55
Group II	2.88	0.87	1.40	0.04
Group III	3.77	2.03	2.57	0.07

little as 0.045 µmoles of either Et-FA or Me-*p*CA to a reaction mixture containing 0.15 µmoles of SA increased the rate of reaction by 30 to 50 times (Fig 1).

Conclusion

The cell walls of corn contain a wide range of POD isozymes that can vary in their substrate specificity to some extent. It would not appear, however, that there is a specific POD for the incorporation of sinapyl alcohol monomers into the latter stages of lignification. It does seem plausible that incorporation of hydroxycinnamic acids into the wall may serve a role in transferring radicals to SA allowing their incorporation into lignin.

Impact

A complete understanding of wall enzymes, such as the peroxidases, provides a clear picture of the metabolic role they play in determining the structure and function of plant cell walls. This provides a larger knowledge base from which we can develop appropriate strategies for improving the digestiblity of grasses (such as corn) without losing hardiness, pest resistance, or yield.

Reference

Takahama, U, Oniki T, and Shimokawa, H. 1996 A possible mechanism for the oxidation of sinapyl alcohol by peroxidase-dependent reactions in the apoplast: Enhancement of the oxidation by hydroxycinnamic acids and components of the apoplast.

Table 2. Comparison of POD acitivities isolated from different corn stem internodes and internode regions. Different substrates included ethyl ferulate, Et-FA; methyl *p*-coumarate, Me-*p*CA; coniferyl alcohol, CA; and sinapyl alcohol, SA.

Corn Stem	Activity (µmoles/min)			
internode	ET-FA	Me-*p*CA	CA	SA
7U	45.89	6.18	29.00	1.30
7L	37.06	4.67	27.48	2.44
5U	71.76	8.96	44.53	1.46
5L	57.75	6.88	36.86	1.22
2U	77.84	12.94	49.02	1.87
2L	79.32	9.21	47.24	1.38